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(54) Title: MARMOSET ZONA PELLUCIDA PROTEIN ZP3

(57) Abstract

The invention comprises a novel polypeptide or a fragment thereof having an amino acid sequence specific for marmoset ZP3. It also comprises antibodies to the polypeptides, as well as vaccines for contraception and methods for expressing the polypeptides in a suitable host and diagnostic kits based on the polypeptides. Furthermore the use of the marmoset ZP3 protein sperm interaction as a model for the development of human contraceptives forms part of the invention.

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MARMOSET ZONA PELLUCIDA PROTEIN ZP3.

The invention relates to a polypeptide having marmoset ZP3 sequence or part thereof. The invention also relates to antibodies against this polypeptide and vaccines or diagnostics with the polypeptides or the antibodies and the use of the marmoset ZP3 system as model for the development of human contraceptives.

During the process of fertilization, the first interaction between mammalian gametes is mediated by binding of sperm cells to a species specific receptor on the zona pellucida (ZP) that surrounds the female gamete. The ZP is an extracellular matrix which comprises three glycoproteins, designated ZP1, ZP2 and ZP3, of which ZP3 has been identified as the sperm receptor (reviewed in Wassarman, Development 108, 1-17; 1990).

Numerous in vitro and in vivo studies using both porcine and murine ZP proteins have indicated that ZP3 is an important candidate target antigen in experimental strategies aimed at the development of immunocontraception (Paterson and Aitken, Curr. Opinion in Immunol., 2, 743-747, 1990). The cloning and characterization of the murine ZP3 cDNA by screening of a mouse ovary cDNA expression library with anti-mouse ZP3 antibodies represents an important step towards this end (Ringuette et al., Developmental Biology 127, 287-295; 1988). This has subsequently allowed the elucidation of the hamster and the human ZP3 sequence (Chamberlin, M.E. and Dean, J., Proc. Natl. Acad. Sci. USA, 87, 6014-6018, 1990 and Kinloch et al., Developm. Biol., 142, 414-421, 1990).

The potential of ZP3 for contraception was emphasized by studies that revealed a long term infertility following vaccination of female mice with an oligopeptide derived from the murine ZP3 amino acid sequence (Millar et al., Science 246, 935-938, 1989).

However, for the development of human contraceptive vaccines based on recombinant or synthetic ZP3 polypeptides or parts hereof, however, there is still need of an in vivo test system. Mice are not suited for such a test system because the biological difference between the conception related molecules of the two species, mouse and human, is too large. This is illustrated by the relative lack of homology (67%) between the mouse and the human ZP3 sequence. Furthermore, at the post-translational level there is a substantial difference between mouse and human ZP3, yielding relative molecular weights of ±83 and 55 kD respectively, whereas the amino acid backbone length of both proteins is identical. This difference is due to different N-linked and/or O-linked glycosylation patterns. The object of the invention therefore is to look for a test system in which the conception related molecules closely resemble those of the human.

We have now succeeded in isolating the gene coding for marmoset ZP3 and elucidating its nucleotide sequence. This nucleotide sequence codes for a polypeptide having marmoset ZP3 activity, said polypeptide comprising the amino acid sequence of SEQ ID NO:1. Fragments of the polypeptide according to the invention are also included in the invention, provided that said fragments comprise an amino acid sequence specific for the marmoset ZP3. Such specific amino acid sequences correspond at least to the specific amino acids and their flanking regions in the marmoset

ZP3, said specific amino acids being positioned at, for example, position 26, 32, 38, 39, 43, 52, 54, 63, 69, 77, 84, 145, 182, 183, 187, 195, 252, 253, 257, 305, 323, 333, 340, 342, 343, 345, 347, 348, 371, 376, 377, 383, 387, 388, 392, 398, 399 or 418 of the amino acid sequence of SEQ ID NO:1.

Marmoset ZP3 activity comprises the sperma binding activity, which can be measured in binding assays, and the potential to induce the sperm acrosome reaction. Marmoset ZP3 activity also comprises the antigenicity of marmoset ZP3. The polypeptides according to the invention are purified and isolated polypeptides. Isolated in this respect means free from other marmoset proteins.

The term "polypeptide" refers to a molecular chain of amino acids, does not refer to a specific length of the product and if required can be modified in vivo or in vitro, for example by glycosylation, amidation, carboxylation or phosphorylation; thus inter alia peptides, oligopeptides and proteins are included within the definition of polypeptide.

The term "contraceptive antigenicity" of the polypeptide according to the invention or fragments thereof refers to the antigenicity displayed by said polypeptide or fragment, resulting in antibodies which are directed to the ZP3 protein, thereby interfering with the sperm-oocyte interaction and thus preventing conception.

To our surprise the polypeptides according to the invention show a high homology with the primary amino acid sequence of human ZP3 (91% on primary amino acid sequence). This homology between the polypeptide according to the invention and human ZP3 makes the marmoset ZP3 an ideal canditate for a test system in the development of human contraceptive vaccines based on human ZP3. Next to the homology on the amino acid

level it has also been found that the molecular weight of the glycosylated protein is ± 55kD. This underlines the high degree of correspondence of the marmoset and human ZP3. The contraceptive antigenicity of the polypeptide according to the invention or fragments thereof observed in the marmoset will be highly indicative of the contraceptive antigenicity of the corresponding homologous human ZP3 or fragments thereof in humans. This use as a model system is also referred to in the phrasing "the marmoset ZP3 system". It will be apparent that the marmoset model system with respect to ZP3 can only be optimal indicative for the corresponding human system if autologous marmoset polypeptides are used. This would not be the case if human ZP3 was used in the marmoset.

Furthermore, due to their high degree of homology with human ZP3 the polypeptides according to the invention are suitable as candidates for a human vaccine. It can be assumed that these polypeptides have maintained the same antigenicity as the corresponding human polypeptides, yet that they have an enhanced immunogenicity while they are still marmoset-specific, i.e. 'foreign' for the immune system of the human body. Such an enhanced immunogenicity would decrease the dose needed for a vaccine to be effective.

The polypeptides according to the invention can be produced either synthetically or by recombinant DNA technology. Methods for producing synthetic polypeptides are well known in the art and do not need any further elaboration.

Production of polypeptides by recombinant DNA techniques is a general method which is known, but which has a lot of possibilities all leading to somewhat different results. The polypeptide to be expressed is coded for by a DNA sequence or more accurately by a nucleic acid sequence.

The nucleic acid sequence coding for the polypeptide according to the invention and fragments thereof is also included in the present invention. Said nucleic acid sequence comprises the nucleic acid sequence of SEQ ID NO:2 or fragments thereof, said fragments coding for marmoset specific ZP3 amino acid sequences as described above. This opens the possibility to produce ZP3 polypeptides and/or ZP3 polypeptide fragments either by recombinant DNA technology or solid phase polypeptide synthesis.

As is well known in the art, the degeneracy of the genetic code permits substitution of bases in a codon resulting in another codon still coding for the same amino acid, e.g. the codon for the amino acid glutamic acid is both GAT and GAA. Consequently, it is clear that for the expression of a polypeptide with an amino acid sequence shown in SEQ ID NO:1 use can be made of a derivate nucleic acid sequence with such an alternative codon composition thereby differing from the nucleic acid sequence shown in SEQ ID NO:2.

The nucleic acid sequence must be transcribed (optionally) and translated to the wanted polypeptide. In order to reach that goal the nucleic acid sequence is normally cloned into a vector with which a host cell is transformed or transfected. A vector is a DNA molecule which is capable to contain heterologous DNA and which can be used to transform or transfect cells. Small plasmids, bacteriophages, viruses, or parts thereof or tailored constructions with fragments of these molecules are often used as vector.

Next to insertion into vectors which are suitable for transporting the DNA into a host cell it is also possible to insert the nucleic acid sequence into a life recombinant carrier.

Different host cells lead to different polypeptides. Prokaryotes do not possess the organelles necessary for glycosylation. The polypeptides produced by them will be without carbohydrate side chains. Eukaryotes do have the glycosylation machinery, but yeast cells will give a different glycosylation pattern than mammalian cells.

Preferred for the polypeptides according to the invention is an expression system which gives the most "natural" glycosylation pattern. Towards this end mammalian cells are most preferred.

Another part of the invention are immunocontraceptive vaccines comprising a polypeptide of the invention. It will be clear to one skilled in the art that the polypeptide of the invention can be bound to a carrier molecule to enhance the immunogenicity. Examples of pharmaceutically acceptable carriers or diluents useful in the present invention include stabilizers such as SPGA, carbohydrates (e.g. sorbitol, mannitol, starch, sucrose, glucose, dextran), proteins such as albumin, keyhole limpet heamocyanin or casein, protein containing agents such as bovine serum or skimmed milk and buffers (e.g. phosphate buffer).

Optionally, one or more compounds having adjuvant activity may be added to the vaccine. Suitable adjuvants are for example aluminium hydroxide, phosphate or oxide, oil-emulsions (e.g. of Bayol $F^{(R)}$ or Marcol $52^{(R)}$, saponins or vitamin-E solubilisate.

The useful dosage to be administered will vary depending on the age, weight and mode of administration.

These vaccines can be used in either a passive or an active immunization.

For an active immunization a polypeptide according to the invention is administered to a female marmoset. The administration will give rise to an immune response by the female. Antibodies will be produced which recognize ZP3 on the ovum. These antibodies either will specifically bind to the sperm binding site or bind to other regions of the ZP3, thus preventing the binding of the spermatozoa.

Passive immunization basically has the same effect. Instead of the antigen or its mimicry the antibodies against it are directly administered. Thus, antibodies have to be raised against the polypeptides according to the invention. Antibodies raised against the polypeptide according to the invention and fragments thereof are also included in the present invention.

This is achieved through an active immunization scheme of a suitable mammal, preferably a mouse or a marmoset. The B-lymphocytes of the mammal are harvested after a suitable period of time and immortalized through fusion or transformation. These methods are well known in the art. Antibodies can be isolated from the culture of the immortalized lymphocytes.

Immunization can also be achieved by infection with a live recombinant carrier which carries the nucleic acid sequence of the invention and which is able to express that sequence. As carriers modified viruses or bacteriae which are normally infectious to mammals can be used. Examples are vaccinia viruses, herpesviruses, adenoviruses, retroviruses and E. coli.

In this way it is possible to obtain antibodies or to test antibodies directed to the sperm receptor on the marmoset oocyt.

Not only the polypeptide itself can be used as an antigen for the production of antibodies. Also antibodies against the carbohydrate residues associated with the ZP3 polypeptides of the invention can be raised. The epitopes for this kind of antibodies can be residing completely in the carbohydrate residues or they can be a combination of carbohydrates and polypeptide backbone. It is of course understood that contraceptive activity of these kind of antibodies can be achieved by active or passive immunization.

Thus the marmoset provides an ideal testing system in the search for contraceptives based on (parts of) the ZP3 sequence. Human homologues corresponding to the marmoset ZP3 epitopes found to be effective in the marmoset female will presumably show similar activity in the human female.

Another possibility is that marmoset ZP3 or fragments thereof or antibodies raised against marmoset ZP3 or fragments thereof in the marmoset, or in an other mammal, show contraceptive activity in human. This can lead, however, to a problem which is encountered with antibodies of animal origin. Upon repeated administration non-human antibodies will give rise to an anti-antibody response in the immunized woman. It is therefore preferred to use either humanized antibodies or small parts of antibodies which will not lead to an immune response. Methods for humanizing antibodies, such as CDR-grafting, are known (for instance Jones et al., Nature 321, 522-525, 1986). Methods for producing fragments of antibodies which are still specific for the antigen of the original antibody are also known (for instance Udaka et al., Molec. Immunol. 27, 25-35; 1990). Another possibility to avoid antigenic response to antibodies to polypeptides according to the invention is the use

of human antibodies or fragments or derivatives thereof.

Human antibodies can be produced by in vitro stimulation of isolated B-lymphocytes, or they can be isolated from (immortalized) B-lymphocytes which have been harvested from a female immunized with the polypeptide of the invention or with the human homologue of such a polypeptide.

Another object of the invention is the use of marmoset ZP3 protein and antibodies directed to it in diagnostic test kits. Diagnostic tests can be applied for many different purposes.

A possible use is found in a sperm function test. ZP3 glycoproteins act as a natural agonist for the acrosome reaction. When applying the glycoprotein of the invention the functionality of sperm samples can be detected. Furthermore, the polypeptides of the invention can be used in monitoring the effect of contraceptive immunization, for instance by detecting circulating antibodies.

Next, labeled antibodies can be used for imaging follicles in the follicle pool, which can be an advantage for the detection of follicles for fertilization schemes such as IVF. In this same respect labeled antibodies can also be used to estimate the number of primordial follicles in the diagnosis of premature ovarian failure.

Another use can be found in the detection of ZP3 related autoimmune diseases. Sera can be screened on the occurrence of autoantibodies against ZP3.

Further, diagnosis of tumours producing ZP3 can be performed with the compounds of the invention. When tumours are present it is also possible to treat this kind of tumours with cytotoxic immunoconjugates based on antibodies against ZP3.

It lies within the skill of the art to produce anti-idiotype antibodies which recognize the antigen binding site of the antibody and therefore are an "internal image" of the antigen. These anti-idiotype antibodies will be also very useful for development of vaccines and for diagnostic purposes.

In the following experimental part the isolation of the marmoset ZP3 DNA-sequence and the marmoset ZP3 amino acid sequence, as well as a method of expressing a recombinant polypeptide showing ZP3 antigenicity, as well as a procedure to make a synthetic polypeptide showing such antigenicity are shown. These examples are merely meant to illustrate the invention and should not be constructed as a limitation of its scope.

EXAMPLE 1.

Construction of cDNA.

Total ovarian RNA was isolated from frozen marmoset ovaries and the poly(A)$^+$ fraction was purified by using oligo(dt) magnetic beads (Dynabeds$^{(R)}$, Dynal). cDNA was synthesized from the purified poly(A)$^+$ RNA using a cDNA synthesis kit (cDNA synthesis kit, Amersham) and then used as a template for amplification of the marmoset ZP3 cDNA using the polymerase chain reaction.
On the basis of the human ZP3 sequence (Chamberlin and Dean, Proc. Natl. Acad. Sci. USA, 87, 6014-6018, 1990) oligonucleotide primers of exon 1 (SEQ ID NO:3) and exon 6 (SEQ ID NO:4) were used (2 min initial melt at 94°C, then 35 cycles; 94 oC 1 min, 60°C 2 min and 72°C 3 min followed by a final extension at 72°C for 15 min). This yielded a 0.8 kbp fragment was purified from agarose gel (Geneclean, Bio 101) and cloned into

the PCR 1000 vector (TA cloning kit, Invitrogen) yielding plasmid pMARZP31-6. The marmoset ZP3 cDNA corresponding to human ZP3 exons 4-8 were also amplified using said human ZP3 sequence, for which the oligonucleotide primer of exon 4 (SEQ ID NO: 5) and exon 8 (SEQ ID NO: 6) were used (30 cycles with annealing temperature of 55°C). This yielded a 0.6 kbp cDNA fragment which was also purified from agarose gel and cloned into the PCR 1000 vector yielding pMARZP34-8.

Sequencing of the PCR 1000 derived marmoset ZP3 cDNA fragments was performed using dideoxy chain termination method (Sequenase, USB) with some modification. Dimethyl sulphoxide (DMSO) was added to termination mixes at a ratio of 9:1 (mix:DMSO) and also to the dilute labelling mix in the same ratio (Winship, P.R., Nucl. Acid Res. 17, 1266, 1989). A complete marmoset cDNA was assembled by ligating a the SalI-SacI fragement of pMARZP34-8 into plasmid pMARZP31-6 opened with SalI and SacI. The plasmid carrying the complete marmoset ZP3 cDNA was designated pMARZP3.

Expression of recombinant ZP3 in CHO cells

For expression of marmoset ZP3 in Chinese hamster ovary (CHO) cells the marmoset ZP3 cDNA from plasmid pMARZP3 has been inserted in a mammalian expression vector in which the ZP3 transcription is driven by the strong SV40 early promoter. In addition to the ampiciline resistance gene, this vector harbors ß-globin splicing and SV40 polyadenylation signals and the selectable marker gene encoding for aminoglycosyl phosphoribosyl transferase (Colbere Garapin et al. J. Mol Biol. 150, 1-14, 1981) which allows for selection of stable transformants using the antibiotic geneticin (Gibco).

In Figure 1 a Western blot analysis is shown of 40x concentrated culture medium of 5 pools of geneticin resistant CHO transformants with a rabbit antiserum raised against human zonae pellucidae proteins. In all 5 samples specific staining of recombinant marmoset ZP3 is detected (lanes 1 to 5) whereas in concentrated culture medium of non-transfected CHO cells no immunostaining is observed (lane 6). Molecular weight markers (M) are given in kDa.

Expression of recombinant marmoset ZP3 in E. coli.

The EcoRI fragment of pMARZP3 has been subcloned in the EcoRI site of the prokaryotic expression vector pMAL-c (New England Biolabs) to allow expression of a fusion protein encoded by a hybrid gene consisting of the E.coli MalE gene (which codes for Maltose Binding Protein, MBP) and the total marmoset ZP3 cDNA sequence. The pMAL-c vector contains an inducible pTac promoter, which allows induction of fusion protein expression with IPTG (isopropyl-ß-D-galactoside). Figure 2 shows a western blot of E.coli expression fusion protein detected with an antiserum against peptide 341-360 of the human ZP3 amino acid sequence. Molecular weight markers (M) are given on the left in kDa. In lane 1 and lane 2 soluble E.coli proteins before and after (3 hours) induction with IPTG are loaded, respectively. In lane 2 the MBP-marmoset ZP3 (MBP-marZP3) fusion protein could be readily detected with this anti human ZP3 peptide antiserum, even though the amino acid 341-360 of marmoset and human ZP3 are not 100% identical. Apparently this peptide harbours epitopes that are conserved between both species.

Legends to the figures

Fig. 1. Western blot analysis of expression of recombinant ZP3 in CHO cells.

Fig. 2. Western blot analysis of expression of recombinant ZP3 in E. coli.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: AKZO N.V.,

(ii) TITLE OF INVENTION: Marmoset zona pellucida protein ZP3

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: AKZO Pharma
(B) STREET: PO Box 20
(C) CITY: Oss
(E) COUNTRY: The Netherlands
(F) ZIP: 5340 BH

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (0)4120-66379
(B) TELEFAX: (0)4120-50592

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 424 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Callithrix jacchus
(F) TISSUE TYPE: Ovary
(G) CELL TYPE: Oocyte

(vii) IMMEDIATE SOURCE:
(B) CLONE: pMARZP3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Glu Leu Ser Tyr Arg Leu Phe Ile Cys Leu Leu Leu Trp Gly Ser
1 5 10 15

Thr Glu Leu Cys Tyr Pro Gln Pro Leu Arg Leu Leu Gln Gly Gly Thr
20 25 30

Ser His Pro Glu Thr Ala Leu Gln Pro Val Val Val Glu Cys Gln Glu
35 40 45

Ala Thr Leu Val Val Thr Val Ser Lys Asp Leu Phe Gly Thr Arg Lys
50 55 60

Leu Ile Arg Ala Val Asp Leu Thr Leu Gly Pro Glu Gly Cys Glu Pro
65 70 75 80

Leu Val Ser Thr Asp Thr Glu Asp Val Val Arg Phe Glu Val Gly Leu
85 90 95

His Glu Cys Gly Asn Ser Met Gln Val Thr Asp Asp Ala Leu Val Tyr
100 105 110

Ser Thr Phe Leu Leu His Asp Pro Arg Pro Val Gly Asn Leu Ser Ile
115 120 125

Val Arg Thr Asn Arg Ala Glu Ile Pro Ile Glu Cys Arg Tyr Pro Arg
130 135 140

Arg Gly Asn Val Ser Ser Gln Ala Ile Leu Pro Thr Trp Leu Pro Phe
145 150 155 160

Arg Thr Thr Val Phe Ser Glu Glu Lys Leu Thr Phe Ser Leu Arg Leu
165 170 175

Met Glu Glu Asn Trp Ser Thr Glu Lys Arg Thr Pro Thr Phe His Leu
180 185 190

Gly Asp Val Ala His Leu Gln Ala Glu Ile His Thr Gly Ser His Val
195 200 205

Pro Leu Arg Leu Phe Val Asp His Cys Val Ala Thr Pro Thr Pro Asp
210 215 220

Gln Asn Ala Ser Pro Tyr His Thr Ile Val Asp Phe His Gly Cys Leu
225 230 235 240

Val Asp Gly Leu Thr Asp Ala Ser Ser Ala Phe Gln Ala Pro Arg Pro
245 250 255

Arg Pro Asp Thr Leu Gln Phe Thr Val Asp Val Phe His Phe Ala Asn
260 265 270

Asp Ser Arg Asn Met Ile Tyr Ile Thr Cys His Leu Lys Val Thr Leu
275 280 285

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Ala Glu Gln Asp Pro Asp Glu Leu Asn Lys Ala Cys Ser Phe Ser Lys
    290                 295                 300

Ala Ser Asn Ser Trp Phe Pro Val Glu Gly Pro Ala Asp Ile Cys Gln
305                 310                 315                 320

Cys Cys Ser Lys Gly Asp Cys Gly Thr Pro Ser His Ala Arg Arg Gln
                325                 330                 335

Pro His Val Val Ser Leu Gly Ser Gly Ser Pro Ala Arg Asp Arg Arg
            340                 345                 350

His Val Thr Glu Glu Ala Asp Val Thr Val Gly Pro Leu Ile Phe Leu
        355                 360                 365

Asp Arg Thr Gly Asp His Glu Met Glu Gln Trp Ala Leu Pro Ala Asp
    370                 375                 380

Thr Ser Leu Leu Leu Leu Gly Thr Gly Leu Ala Val Val Ala Leu Leu
385                 390                 395                 400

Thr Leu Thr Ala Val Ile Leu Val Leu Thr Arg Arg Cys Arg Thr Ala
                405                 410                 415

Ser Leu Pro Val Ser Ala Ser Glu
            420
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1275 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Callithrix jacchus
(F) TISSUE TYPE: Ovary
(G) CELL TYPE: Oocyte

(vii) IMMEDIATE SOURCE:
(B) CLONE: pMARZP3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ATGGAGCTGA GCTATAGGCT ATTCATCTGC CTCCTGCTCT GGGGTAGTAC ATGAGCTGTGC 60

TACCCCCAAC CCCTCAGGCT CTTACAGGGT GGAACCAGCC ACCCTGAGAC CGCTCTGCAG 120

CCCGTAGTGG TGGAGTGTCA GGAAGCCACC CTAGTGGTCA CAGTCAGTAA AGACCTTTTT 180

GGCACCAGGA AGCTTATCAG GGCTGTTGAT CTCACCCTGG GCCCAGAGGG CTGTGAGCCC 240

CTGGTCTCCA CGGACACAGA GGATGTGGTC AGGTTTGAGG TTGGACTCCA TGAGTGTGGT 300

AACAGCATGC AGGTGACCGA CGATGCCCTG GTGTACAGCA CCTTCCTGCT TCACGACCCC 360

CGCCCTGTGG GAAACCTGTC CATCGTGAGG ACTAACCGCG CAGAGATTCC CATCGAGTGC 420

CGCTACCCCA GGCGGGGCAA TGTGAGCAGC CAGGCCATCC TTCCCACCTG GCTGCCCTTC 480

AGGACCACGG TGTTCTCAGA GGAGAAGCTG ACTTTCTCTC TGCGCCTGAT GGAGGAGAAC 540

TGGAGCACTG AGAAGAGGAC CCCTACCTTC CACCTGGGAG ATGTGGCCCA CCTCCAGGCA 600

GAAATCCACA CTGGCAGCCA CGTGCCACTG CGGCTATTTG TGGACCACTG TGTGGCCACG 660

CCAACACCAG ACCAGAATGC CTCCCCTTAT CACACCATCG TGGACTTCCA TGGCTGTCTT 720

GTCGACGGTC TCACTGATGC CTCTTCTGCA TTCCAAGCTC CCAGACCCAG GCCAGATACA 780

CTCCAGTTCA CGGTGGATGT GTTTCATTTT GCTAATGACT CCAGAAATAT GATATACATC 840

ACCTGCCACC TGAAGGTCAC CCTAGCTGAG CAGGACCCAG ATGAACTGAA CAAAGCCTGT 900

TCCTTCAGCA AGGCTTCCAA CAGCTGGTTC CCGGTGGAAG GCCCGGCTGA CATCTGCCAG 960

TGCTGTAGCA AGGGTGACTG TGGCACTCCA AGCCATGCCA GGAGGCAGCC CCATGTCGTG 1020

AGCCTGGGGT CGGGTTCTCC TGCCCGTGAC CGCAGGCATG TGACAGAAGA AGCAGACGTC 1080

ACCGTGGGAC CGCTGATCTT CCTGGACAGG ACTGGTGACC ACGAAATGGA GCAGTGGGCC 1140

TTGCCGGCTG ACACCTCCTT GCTGCTGCTG GGCACAGGCC TGGCTGTTGT GGCGCTCCTG 1200

ACTCTGACCG CTGTTATCCT GGTTCTCACC AGGAGGTGTC GCACTGCCTC CCTCCCTGTG 1260

TCTGCTTCCG AATAA 1275

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TGCAGGGTAC CATGGAGCTA TAGGC 25

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CAGGTGGCAG GTGATGTA 18

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATCACACCAT CGTGGAC 17

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGACACAGAC GAAGGCTTAT TTTCTTCCTT AAGCGC 36

CLAIMS

1) Polypeptide having marmoset ZP3 activity, characterized in that said polypeptide comprises the amino acid sequence of SEQ ID NO:1, or fragments thereof, said fragments comprising an amino acid sequence specific for marmoset ZP3.

2) Polypeptide according to claim 1, characterized in that said polypeptide is at least partially glycosylated.

3) Nucleic acid sequence coding for a polypeptide according to claim 1 or 2.

4) Nucleic acid sequence according to claim 3, characterized in that it comprises the nucleic acid sequence of SEQ ID NO:2, or fragments thereof, said fragments coding for amino acid sequences specific for marmoset ZP3.

5) A vector comprising a nucleic acid sequence according to claim 3 or 4.

6) Live recombinant carrier comprising a nucleic acid sequence according to claim 3 or 4.

7) A host cell transformed or transfected with a nucleic acid sequence according to claim 3 or 4 or a vector according to claim 5.

8) An immunocontraceptive vaccine comprising a polypeptide according to claim 1 or 2.

9) An immunocontraceptive vaccine according to claim 8 comprising in addition one or more polypeptides having ZP3 activity from other mammals.

10) Antibodies raised against a polypeptide according to claim 1 or 2.

11) An immunocontraceptive vaccine comprising an antibody according to claim 10.

12) A method for producing a polypeptide according to claim 1 or 2, comprising transformation or transfection of a host cell with a vector comprising a nucleic acid sequence coding for such a polypeptide, culturing said host cell in a suitable medium and isolating the polypeptide from the culture.

13) A method for producing an antibody according to claim 10, characterized in that a suitable animal is injected with a polypeptide according to claim 1 or 2, and that the B-lymphocytes of said animal are harvested after a suitable period of time and that antibody producing cells are selected and immortalized, after which they are cultured and the antibodies are isolated from the culture.

14) A diagnostic composition comprising a polypeptide according to claim 1 or 2 or an antibody according to claim 10.

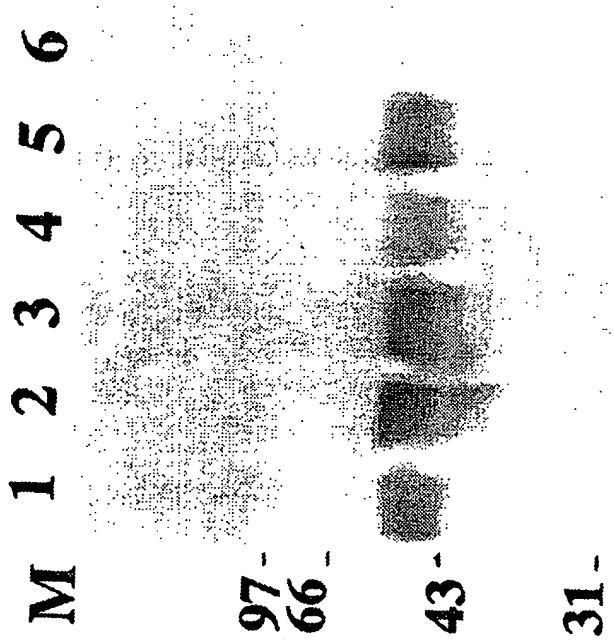

Fig 1

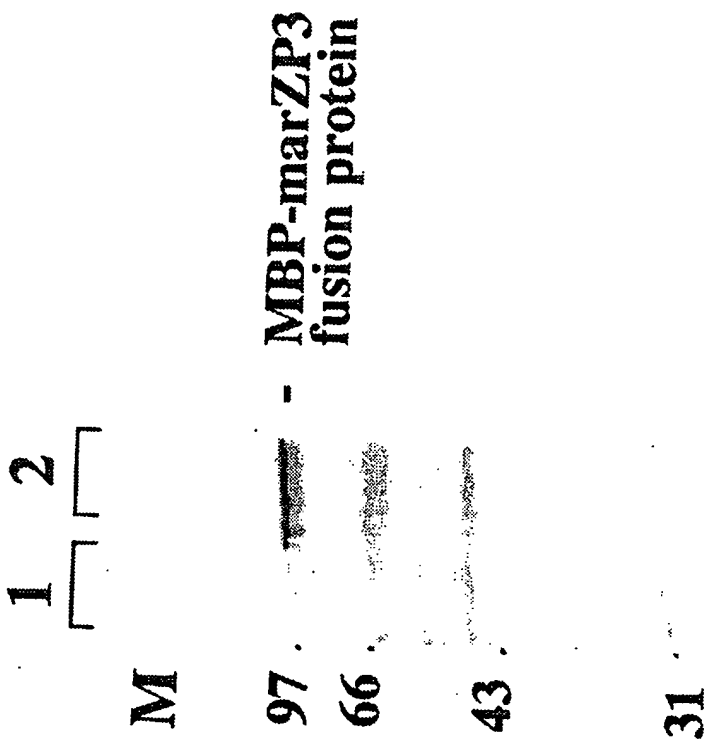

Fig 2.

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/EP 93/03014

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/12 C07K13/00 A61K39/00 G01N33/577 C12P21/08
C12N5/10 C12N1/21 //(C12N1/21,C12R1:19)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12N C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,92 03548 (AKZO NV, NL) 5 March 1992 see the whole document ---	1-14
X	WO,A,90 15624 (THE UNITED STATES OF AMERICA) 27 December 1990 see the whole document ---	1-14
X	BIOLOGY OF REPRODUCTION vol. 46, no. 4 , April 1992 pages 523 - 534 PATERSON, M. ET AL.; 'Analysis of the contraceptive potential of antibodies against native and deglycosylated porcine ZP3 in vivo and in vitro' See the discussion --- -/--	10,11

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 January 1994	11 -02- 1994

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	NAUCHE, S

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FERTILITY AND STERILITY vol. 56, no. 4 , October 1991 pages 764 - 767 Liu DY;Lopata A;Pantke P;Baker HW; 'Horse and marmoset monkey sperm bind to the zona pellucida of salt-stored human oocytes.'	

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internal Application No
PCT/EP 93/03014

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9203548	05-03-92	AU-A-	8328591	17-03-92
		CA-A-	2090486	28-02-92
		CN-A-	1060499	22-04-92
WO-A-9015624	27-12-90	AU-B-	636895	13-05-93
		AU-A-	5826790	08-01-91
		CA-A-	2058999	13-12-90
		EP-A-	0477226	01-04-92
		JP-T-	5500654	12-02-93